CALIFORNIA DEPARTMENT OF FISH AND GAME
WATER POLLUTION CONTROL LABORATORY
AQUATIC BIOASSESSMENT LABORATORY
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QUALITY ASSURANCE PROJECT PLAN FOR THE CALIFORNIA STREAM BIOASSESSMENT PROCEDURE

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PROBLEM DEFINITION AND BACKGROUND

California's streams and lakes provide essential habitat for freshwater plants and animals and provide important recreation opportunities. Identifying unique aquatic habitats, recognizing endemic species of plants and animals and assessing whether our streams and lakes are healthy or impaired is an important part of water resource management. However, California water resource agencies currently do not provide sufficient data to document the physical and biological condition of the state's water bodies.

Bioassessment, a tool for measuring stream water and habitat quality based on the kinds of organisms living there, has recently been implemented in California with the goal of incorporating biological criteria into water quality standards. Such criteria can be used to protect biological resources, report on the condition of water bodies, identify impaired water bodies and set restoration goals for impaired sites. In fact, the Clean Water Act mandates that "States shall adopt [water quality] criteria based on biological monitoring or assessment methods" (Section 303(c)(2)(B)), and that "States shall develop and publish criteria for water quality accurately reflecting the latest scientific knowledge... on the effects of pollutants on biological community diversity, productivity and stability" (Section 304 (a)(1)).

Only in recent years, with encouragement from the EPA, have states started to collect adequate data on the physical and biological health of their water bodies. In 1993, California initiated the first step in developing a state-wide bioassessment program by introducing the California Stream Bioassessment Procedure (CSBP). The CSBP is a standardized protocol for assessing biological and physical/habitat conditions of wadeable streams in California, and is a regional adaptation of the national Rapid Bioassessment Protocols outlined by the U.S. Environmental Protection Agency (EPA 841-D-97-002). The CSBP is a cost-effective tool that utilizes measures of a stream's benthic macroinvertebrate (BMI) community and its physical/habitat characteristics to determine the stream's biological and physical integrity. BMIs can have a diverse community structure with individual species residing within the stream for a period of months to several years. They are also sensitive in varying degrees to temperature, dissolved oxygen, sedimentation, scouring, nutrient enrichment and chemical and organic pollution. Biological and physical assessment measures integrate the effects of water quality over time, are sensitive to multiple aspects of water and habitat quality and can provide the public with a familiar expression of ecological health. Now in its third edition, the CSBP is recognized as California's standard protocol for conducting physical and biological surveys, and forms the basis of California's effort to develop a state-wide bioassessment program (Davis et al. 1996).

Bioassessment studies can normally be divided into two types: ambient monitoring and point source monitoring. Ambient monitoring consists of regular sampling within watersheds to establish baseline (i.e., current) conditions so that future changes can be evaluated for compliance with legally mandated water quality standards. Point-source monitoring involves surveys done before and after (or upstream and downstream) of a specific impact to determine the effects of that impact on aquatic communities.

In either case, our ability to recognize degradation at potentially damaged sites relies on our understanding of conditions expected in the absence of disturbance. In point-source monitoring, biological conditions observed upstream of an impact can be used as a reliable indicator in defining community recovery downstream. In ambient monitoring, expected conditions must usually be inferred from "reference sites". In some studies, it is possible to select sites that have experienced minimal human impact and thus reflect pristine conditions. In areas where human alteration of the landscape is significant, reference sites are likely to be "least impaired sites", and thus reflect the best conditions possible given the extent and duration of human activity (Hawkins et al., 2000).

The DFG-ABL has collected bioassessment data for thousands of stream study sites in California since 1993. The objectives of the bioassessment program outlined herein are as follows:

1) Establish appropriate biocriteria (e.g., Indexes of Biological Integrity, IBI's) on a regional basis by defining reference stream conditions for different stream types in each of California's ecoregions. Regional reference conditions are essential for assessing the status of streams that may have degraded ecological integrity, and are also essential in monitoring the success of restoration efforts.

2) Provide field and analytical support for California's Regional Water Quality Control Boards as they incorporate ambient biological monitoring into water quality management. In addition to establishing regional IBI's, this includes developing lists of BMI's known from various regions, determining the best index periods for sampling BMI's and identifying highly impacted streams so that restoration efforts can be prioritized. Examples include ongoing projects for the San Diego and Bay Area Regional Water Quality Control Boards.

3) Evaluate the biological integrity of streams exposed to various point-source impacts (enforcement cases). Site-specific baseline data are used to monitor the success of management actions taken.

4) Offer technical support for other agencies conducting bioassessment in California by establishing standardized field and laboratory protocols, taxonomic expertise and Quality Assurance/Quality Control services.

5) Comparison and calibration of various bioassessment sampling protocols (e.g., CSBP, RIVPACS, EMAP).

PROJECT/TASK DESCRIPTION

DATA QUALITY OBJECTIVES

Data collected will allow assessment of the integrity of BMI communities throughout California, and will thereby facilitate the development of water quality criteria and the evaluation of impaired water bodies in the context of those criteria. Data from reference sites, or minimally impaired sites in highly impacted areas, is essential in the development of biocriteria. The total number of sites sampled, including the number of minimally impacted sites required to establish reference conditions and the number of impacted sites that will require future monitoring to assess compliance with legally mandated water quality objectives, will vary depending on the scope and goals of individual projects.

The CSBP targets BMI communities that occur in riffle habitats within streams. Riffles are the most productive microhabitats within in a stream in terms of taxonomic diversity, and sampling within a specific microhabitat facilitates comparison of community composition between streams (such comparisons can be confounded by multi-habitat sampling approaches when study streams vary significantly in types of habitat available). In order to reduce the likelihood of inaccurate assessment of benthic communities due to sampling error and the "patchiness" that often characterizes the distribution of BMI's, the CSBP utilizes triplicate measures of BMI community composition by sampling three different riffles within a stream reach.

BMI communities should be sampled when streams are at base flow and before mass seasonal emergences have occurred (sampling index periods will vary with region). Furthermore, evaluation of BMI community composition relies on accurate taxonomic identifications and a standard level of identification (e.g., genus) that allows discrimination among sites. A guidance document outlining the desired level of taxonomic effort has been established by the California Aquatic Bioassessment Laboratory. Because accurate taxonomy is imperative in bioassessment, a minimum of 95% accuracy in taxonomic identifications is required. Taxonomic accuracy is evaluated according to the QC protocol outlined below.

Assessment of physical habitat quality is inherently more subjective than taxonomic identification. The QA procedure outlined below has been designed to maximize accuracy and consistency in physical habitat assessment. Error rates of approximately 25% are considered acceptable for physical habitat assessment, i.e., independent evaluations of physical habitat at a given site should rank the site in the same broad quality category, of which there are four (see the attached sheet that outlines physical habitat parameters assessed per site).

SPECIAL TRAINING REQUIREMENTS

All ABL staff members are trained in the use of the protocols outlined below. New field staff members are trained by experienced members or by project managers. Before each field season, all staff members are involved in training sessions to review protocols used in physical habitat, chemical and biological surveys. These training sessions involve practice sampling and habitat assessment.

Most of the taxonomists in the lab have graduate degrees (M.S. or Ph.D.) in Entomology or Ecology, and have many years of experience in invertebrate taxonomy and identification. Lab technicians receive training and direct oversight from taxonomists.

DOCUMENTATION AND RECORDS

All field data (physical habitat and water chemistry measurements) are entered on standardized forms that are completed at the time of sampling (see attached forms). Laboratory records (e.g., COC's and sample processing information) are also standardized (see attached forms) and are kept in clearly labeled files in the ABL lab. Lists of all identified taxa and the ABL Sample Inventory are stored by project in CAL EDAS, an ACCESS© data base that facilitates the archiving and retrieval of taxonomic information. Vouchers of all identified BMI's are kept for every project, and a reference collection of macroinvertebrates found in California has been established.

SAMPLING PROCESS DESIGN

Water quality assessment on a watershed basis requires at least one or two years of sampling effort by a water resource agency. This allows the establishment of baseline physical and biological information, and allows the development of a reference framework (IBI) to assess the present and long-term condition of water resources within the watershed. Our ability to accurately characterize the biological integrity of sites, and thereby quantify impairment when it exists, relies on the development of this framework.

Sample Site Selection: The primary goal in selecting sampling sites is to represent the major stream systems, ecoregion subsections, vegetation zones, stream orders and elevations within the watershed. Factors that may limit the number of study reaches include accessibility (physical and legal) and suitable riffle habitat. Land ownership throughout the watershed can limit site selection to areas where written permission is granted. Land owners bordering potential sampling sites can be identified through the local county assessors office, and contacts with property owners should utilize a standardized form. Sample site selection should include input form the Watershed Advisory Group and any public land agency located in the watershed.

Reach length depends on the frequency of riffle/run habitat units and uniformity of channel type. The objective in BMI sampling is to establish reaches that contain at least five riffle/run habitat units within the same channel type. If the reach length is limited by private land, at least three riffle/run habitat units should be delineated.

A Global positioning system (GPS) is used to determine the coordinates of the sites whenever possible. Manual mapping of the sites is also done using major landmarks, 7.5 minute USGS (1:24,000 scale) and USFS topographical maps. Latitude and longitude are determined to the nearest second.

Once sampling sites are determined the following generalized tasks are performed:

1) Summarize historic data and published information on watershed (non-critical)
2) Determine site ownership (non-critical)
3) Contact owners for access approval and education (non-critical)
4) Describe site location in detail including GPS (non-critical)
5) Photo-document study reaches (non-critical)
6) Define study reach through channel typing (critical)
7) Describe watershed characteristics (critical)
8) Measure habitat integrity using the CSBP (critical)
9) Measure biological integrity (critical)
10) Measure ambient water chemistry (critical)
    - temp, DO, conductivity, turbidity, pH
11) Determine land-use activities (non-critical)
12) Build GIS overlays of gathered information (non-critical)
13) Rank areas of watershed degradation (critical)
14) Test bioassessment metrics using land-use activities and ranking (critical)
15) Validate CSBP bioassessment metrics (critical)
    - within reaches
    - between reaches
    - seasonality
16) Test available biotic indices (Moyle IBI, EPA, CSBP) (critical)
17) Develop framework for biotic index for watershed (critical)
18) Finalize data analysis and reduction (critical)
19) Make project description and data available on CABW homepage (non-critical)
20) Train and integrate watershed advisory group into a long-term monitoring program (critical)

SAMPLING METHODS REQUIREMENTS

Field Procedures for Collecting BMI Samples and Assessing Physical Habitat Quality

The CSBP can be used to detect aquatic impacts from point and non-point sources of pollution and for assessing ambient biological condition. The sampling unit is an individual riffle or riffles within a reach of stream depending on the type of sampling design used. Riffles are used for collecting biological samples because they are the richest habitat for BMIs in wadeable streams. **The BMI sampling procedures described in this Protocol Brief are intended for sampling wadeable, running water streams with available riffle habitats.** There are approved modifications of this procedure for narrow (< 1m) streams, wadeable streams with sand or mud bottoms and channelized streams. There are also procedures for lentic or still water environments. Contact DFG or visit the California Aquatic Bioassessment Web Site for more information.

It is important that BMI's are collected when streams are at base flow, as high flows can dramatically alter local community composition and can thus produce unrepresentative results.

Field Equipment and Supplies

- Measuring tape (100 meter)
- D-shaped kick net (0.5mm mesh)
- Standard Size 35 sieve (0.5mm mesh)

Field Equipment and Supplies (continued)

- Wide-mouth 500 ml plastic jars
- White sorting pan and forceps
- 95% denatured ethanol
- California Bioassessment Worksheet (CBW)
- Physical/ Habitat Quality Form
- Flow meter
- Random number table
- pH, temperature, DO and conductivity meter
- Stadia rod and hand level/ clinometer
- Densiometer/ Solar Pathfinder
- GPS unit or watershed topographic map

Point Source Sampling Design

There will be discernable perturbations, impacting structures or discharges into the stream with point sources of pollution. The sampling units will be individual riffles within the affected section of stream and an upstream unaffected section. At least one riffle in the unaffected section should be sampled and one or more riffles in the affected section depending on the amount of detail that is required on downstream recovery. The riffles used for sampling BMIs should have relatively similar gradient, substrate and physical/habitat characteristics and quality. **One sample will be collected from 3 randomly chosen transects in each riffl**e.

Use the following step-by-step procedures for collecting BMIs using the point source sampling design:

Step 1. Place the measuring tape along the bank of the entire riffle while being careful not to walk in the stream. Each meter or 3 foot mark represents a possible transect location. Select 3 transects from all possible meter marks along the measuring tape using a random number table. Walk to the lowest transect before proceeding to Step 2.

Step 2. Inspect the transect before collecting BMIs by imagining a line going from one bank to the other, perpendicular to the flow. Choose 3 locations along that line where you will place your net to collect BMIs. If the substrate is fairly similar and there is no structure along the transect, the 3 locations will be on the side margins and the center of the stream. If there is substrate and structure complexity along the transect, then as much as possible, select the 3 collections to reflect it.

Step 3. After mentally locating the 3 areas, collect BMIs by placing the D-shaped kick net on the substrate and disturbing a 1x2 foot portion of substrate upstream of the kick-net to approximately 4-6 inches in depth. Pick-up and scrub large rocks by hand under water in front of the net. Maintain a consistent sampling effort (approximately 1-3 minutes) at each site. Combine the 3 collections within the kick-net to make one composite sample.

Step 4. Place the contents of the kick-net in a standard size 35 sieve (0.5 mm mesh) or white enameled tray. Remove the larger twigs, leaves and rocks by hand after carefully inspecting for clinging organisms. If the pan is used, place the material through the sieve to remove the water before placing the material in the jar. Place the sampled material and label (see below) in a jar and completely fill with 95% ethanol. Never fill a jar more than 2/3 full with sampled material and gently agitate jars that contain primarily mud or sand.

Bioassessment Sample Label

Watershed: _____
County: _____
Stream Name: _____
Site Code: _____
Date Collected: _____
Collector: _____
Time Collected: _____
Transect/ Replicate: _____

Step 5. Proceeding upstream, repeat Steps 2 through 4 for the next two randomly chosen transects within the riffle.

Non-point Source Sampling Design

There will be no obvious perturbations or discharges into the stream with non-point sources of pollution. This sampling design is appropriate for assessing an entire stream or large section of stream. The sampling units will be riffles within a reach of stream. The stream reach must contain at least 5 riffles within the same stream order and relative gradient. **One sample will be collected from the upstream third of 3 randomly chosen riffles.**

Use the following step-by-step procedures for collecting BMIs using the non-point source sampling design:

Step 1. Randomly choose 3 of the 5 riffles within the stream reach using the random number table.

Step 2. Starting with the downstream riffle, place the measuring tape along the bank of the entire riffle while being careful not to walk in the stream. Select 1 transect from all possible meter marks along the top third of the riffle using a random number table.

Step 3. (See Point Source Sampling Design Step 2)

Step 4. (See Point Source Sampling Design Step 3)

Step 5. (See Point Source Sampling Design Step 4)

Step 6. Proceeding upstream, Repeat Steps 2 through 5 for the next two riffles within the stream reach.

Sampling Design for Assessing Ambient Biological Conditions

Assessment of ambient biological condition utilizes both the point and non-point source sampling designs to cover an entire watershed or larger regional area. Ambient bioassessment programs are used to evaluate the biological and physical integrity of targeted inland surface waters. Stream reaches should be established in the upper, middle and lower portions of each watershed and above and below areas of particular interest. Quite often bioassessment is incorporated into an existing chemical or toxicological sampling design. In most cases, the water quality information is being collected at a particular point on the stream. Although there will be the tendency to use the point source design, try to convert to a non-point reach design for biological sampling.

Measuring Chemical and Physical/Habitat Quality

Measurements of chemical and physical/habitat characteristics are used to describe the riffle environment and help water resource specialists interpret BMI data. The information can be used to classify stream reaches and to explain anomalies that might occur in the data.

The physical/habitat scoring criteria are based on the EPA's nationally standardized methods. They are used to measure the physical integrity of a stream, and can be a stand-alone evaluation or used in conjunction with a bioassessment sampling event. DFG recommends that this procedure be conducted on every reach of stream sampled as part of a bioassessment program. Fill out the Physical/Habitat Quality Form (see attached) for the entire reach where BMI samples are collected as part of a non-point source sampling design. Some of the parameters do not apply to a single riffle, so this procedure is usually not performed as part of the point source sampling design. **This procedure is an effective measure of a stream's physical/habitat quality, but requires field training prior to using it and implementation of quality assurance measures throughout the field season.** A detailed description of the scoring criteria is provided at the end of this document.

A Physical/Habitat Quality Form should be filled out for each individual riffle when following the Point Source Sampling Design and for the entire reach when using the Non-point Sampling Design. Use the following step-by-step procedures for filling out the form:

Step 1. At the top of the form, enter basic information about the sampling event. This includes watershed and stream name, site code (if available) date and time of sample collection, name of the company or agency collecting the samples, names of each crew member, latitude and longitude and elevation.

Step 2. Record the water temperature, specific conductance, salinity, pH and dissolved oxygen measurements in the appropriate place. These measurements should be taken using standardized methods and approved equipment (see above).

Step 3. Estimate or measure the entire length of the reach where the three riffles were chosen as part of the non-point source sampling design. For point-source sampling, estimate or measure the length of the riffle sampled.

Step 4. **For each riffle**:

- Measure the riffle velocity and depth using a flow meter placed in front of the three locations along the transect(s) where the BMI samples were collected. Average the readings.
- Estimate the percent of the riffle surface that is covered by shade from streamside vegetation (canopy cover) using a densiometer at several places along the riffle. Average the readings.
- Measure the length and width of each of the three riffles. If width is not uniform, take several measurements and average them.
- Visually estimate the percent of riffle in each of the following substrate categories: fines (<0.1"), gravel (0.1-2"), cobble (2-10"), boulder (>10") and bedrock (solid). Use the entire riffle to assess this parameter and make note if the area along the transect(s) is considerable different from the rest of the riffle.
- Estimate substrate consolidation by kicking the substrate with the heel of your wader boots to note whether it is loosely, moderately or tightly cemented. The estimate should

take into consideration the hands-on experience obtained from collecting the BMI sample.

- Determine substrate complexity and embeddedness within the riffle (Parameters 1 and 2, respectively, from the Habitat Parameter Guidelines). Use the entire riffle to assess these parameters, and make note if the area along the transect(s) is considerably different from the rest of the riffle.
- Measure the gradient or slope of the riffle using a stadia rod and a hand level or a clinometer.

Step 5. **For the entire reach**:

- Using the Habitat Parameter Guidelines (see attached), estimate items 1-10 on the Physical Habitat Form (epifaunal substrate, embeddedness, etc.) **Note that items 8-10 require a separate estimate for each bank.**
- Draw a map of the reach indicating location of the riffles and any access points on the back of the Physical Habitat Quality Form.

SAMPLE HANDLING AND CUSTODY REQUIREMENTS

Sample Log-in Procedures: **Initial ABL Sample Handling and Chain of Custody (COC) Form:** Each set of samples submitted to the Aquatic Bioassessment Laboratory must be accompanied by a complete COC form (see attached). In most cases samples are collected by ABL staff, but we occasionally receive samples collected by other agencies. Procedures for generating a COC under either circumstance are listed below.

<u>Samples collected by ABL staff</u>

When samples arrive at the ABL the first priority is to log the samples into the electronic ABL Sample Inventory Database (CAL EDAS). The information entered into this database is then used to generate a COC form. The inventory log contains the following information:

1. The project name and the watershed name.
2. **Complete locality information** for each sample, including county where sampling occurred, site description (e.g., Pine Creek at Centerville Road), transect information, sampling date and name of collector.
3. Date and time samples arrived at the ABL.
4. Total number of samples (and total number of jars if different from total samples due to single samples occupying more than one jar).
5. Sample ID numbers ("ML numbers"). These are assigned to each sample during the log in procedure.
6. (**Optional**) Site descriptions for each individual sample (the site description is an abbreviated code derived from the original sampling location; for example, Santa Margarita at Camp Pendleton= SMR-CP).

All samples from a given project are logged in at once so that the ABL numbers generated for that project are consecutive. When more than one watershed is sampled in a project, all samples from each watershed should be grouped so that ABL numbers are consecutive within watersheds. It is desirable to have samples within a watershed logged in according to elevation so that upstream sites receive the lowest numbers in a series.

Once all samples have been logged into the ABL Sample Inventory Database, the sample information is printed as a COC using the Access report function. The COC is signed by one of the ABL staff members. Following completion of the COC form, the appropriate ML number is affixed to each sample container.

Samples collected by other agencies

Samples delivered form other agencies must be accompanied by a COC form at the time of delivery (**note**: a page of instructions for agencies that want to submit samples is attached), and must contain the following information in addition to that listed above:

1. The name of the agency that completed the original sampling, the name of that agencies' project advisor, the name of at least one crew member that participated in sampling, and address/telephone numbers for both.

2. A list of sample ID numbers (***if*** ID numbers have been assigned by the originating agency; otherwise, ID numbers are assigned to each sample during sample log in.

Upon transfer of samples, the presence of each sample listed on the COC form is verified by ABL staff. After verification the relinquisher signs and dates that portion of the COC form titled "Relinquished by" and the ABL lab technician signs and dates the section titled "Received by" to complete this stage of the COC procedure.

All COC forms are kept in a clearly marked file folder in the general files of the ABL. Three separate COC files have been established as follows:

1. QA-QC projects
2. Enforcement cases
3. Standard ambient bioassessment projects

At all times the original COC will accompany the samples. Once subsampling has been completed, the original COC accompanies the subsampled macroinvertebrates, and a photocopy of the COC will remain with the original samples and subsampling remnants. Any time a sample or set of samples is removed from the lab for any reason, the transfer is noted on the appropriate COC, including the date and personal responsible for transfer.

ANALYTICAL METHODS REQUIREMENTS

Subsampling

Find the sample identification label on the sample jar lid and confirm that it matches the sample description label inside the jar and the information recorded in the Macroinvertebrate Sample Inventory Log. Keep track of the location information, as it will be duplicated and used repeatedly in all subsequent steps, and therefore must be accurate.

Place a 2000 ml glass beaker beneath a #35 standard sieve (0.5 mm mesh). Pour contents of sample jar into sieve. Be aware that there may be more than one jar for a sample. Rinse any excess material from sample jar into sieve with tap water. Record the approximate volume of waste alcohol produced during the day on the "Evaporation Pond Chemical Disposal" form. The waste alcohol is dumped down the

sink drain and collects in an evaporation pond. After disposing waste alcohol rinse the sink with tap water to flush the alcohol completely.

Rinse the sample in sieve with tap water to remove any fine particles (<0.5mm). Positioning an enamel tray under the sieve during the washing process serves two functions: (1) it allows the lab technician to determine when the sample is adequately rinsed of fine sediment and (2) organic detritus can be teased apart and more easily distributed throughout the sample if the sieve is placed into a tray full of water, thereby suspending such material. The stage of rinsing should be done **carefully** so damage to organisms is minimized.

After rinsing sample with water, inspect any large rocks (gravel size) or large leaves that have not begun to decompose for attached invertebrates. Clinging invertebrates should be removed and placed in the sieve, after which these larger materials can be placed in a "remnant jar" (see below). *Decayed* leaves and twigs are left in the sieve and should be carefully inspected for invertebrates with the aid of a stereo microscope during subsampling (see below). Drain excess water from the sieve.

Transfer the contents of the sieve to a subdivided 8" X 10" tray (the tray should be subdivided into twenty 25cm^2 squares and numbered so each square can be identified). One technique for transfer is to first quickly invert the sieve over the tray and tap on the sieve to dislodge the material. Then concentrate any sample that remains in the sieve into one portion at one end of the sieve with tap water. This portion may then be rinsed into the tray with small portions of 70% ethanol/ 3% glycerol solution. Inspect the sieve for attached invertebrates and transfer any that are found to the tray with the rest of the material.

While being careful not to damage organisms, distribute the sample evenly throughout the tray so that different material types are dispersed homogeneously. This step is critical to a good subsample, so take several minutes to do it right!

Add enough 70% denatured ethanol/ 3% glycerol solution to the sample so that tray contents are wetted but not completely submerged. **Do not overfill the tray because organisms tend to float to other grids.**

Randomly select at least 5 numbers from 1 to 20 using a random number table, or use some other random number generator. Record these numbers on the ABL Subsampling Worksheet (see attached). These numbers define which grids in the grid tray you will actually sample from.

To begin, divide the material from within the first randomly chosen grid into quarter grids. Quarter grids are made by carefully cutting through or teasing the sample apart diagonally with a one-sided razor blade. Transfer the contents of the first quarter grid to a petri dish using the razor blade.

With the aid of a stereo microscope at a minimum of 10 X magnification, transfer invertebrates from the petri dish to a screw top 25 ml glass vial containing 70% ethanol/ 3% glycerol. This vial should include a proper locality label. The label includes the state, county, sampling site (i.e., name of stream or lake and the point at which it was sampled), replicate # (e.g., T1), date collected, ML number, and field collector initials (see below for additional notes about labels). This label is used in all subsequent handling of the samples.

CA: Tehama Co. 10/19/99
Dry Creek at Stewart Road
ML# 3519 T1
Coll. MD, CS

Count the number of invertebrates removed from the tray with an auto- counter. **If you suspect a counting error, recount.** Note: remove macroinvertebrates from the petri dish in a consistent, uniform manner. Process grids from left to right, top to bottom, and do not remove larger invertebrates first.

Grids are processed until 300 invertebrates are obtained. It is important to subsample from *at least three* different grids in the grid tray. If the first quarter grid contains over 100 organisms, then EIGHTH GRIDS should be used thereafter. If between 50 and 100 organisms are encountered in the first quarter grid, then continue to use quarter grids until 300 organisms have been picked. If very few organisms are encountered in the first quarter grid (e.g., 20 or less), then half grids or even whole grids should be used. Remember, the key is to sample from *at least three* different grids. Obviously samples with high numbers of organisms will require subsampling from fewer total grids before 300 organisms are picked. Always decide which side of the grid you will process before subsampling and be consistent with each grid.

When 300 organisms are obtained before the last grid is completely processed, the remaining organisms in the grid are totaled and transferred to a separate vial with a location label and a label identifying them as "extra bugs". These "extra bugs" are used in the abundance calculations, but are not identified. Record the number of invertebrates removed from each grid on the ABL Subsampling Worksheet.

The following **must not be included** in the invertebrate count and should be placed in the remnant container (if there is any doubt about what to include, consult with a taxonomist):

1) organisms that were dead before sampling (these can be recognized by their generally decayed "husk-like" and frail appearance, and will often lack one or more body parts).
2) exuviae
3) organisms with incomplete bodies (a head, thorax and most of the abdomen should be present)
4) terrestrial invertebrates
5) semi-aquatic insects including Collembola and surface hemipterans
6) worm fragments - this may depend on the project. If oligochaetes are to be identified to family, only heads should be counted, or count heads and tails and divide by two.
7) empty shells and cases (e.g., gastropods, ostracods, clams, caddisflies, chironomids)

Place the residual material from the processed grids into a separate half pint or pint size mason jar. Include a location label. This processed residue is considered the remnant material of the sample. Affix a label to the outside of the remnant jar that includes the ML # and description: "Remnant". The remainder of the original sample material that is left over in the subdivided tray is placed in a half pint or pint size mason jar and labeled as "original". Again, make sure there is a location label inside the mason jar and the outside of the jar is labeled with the correct ML # and description: "original".

Estimated abundance calculation - The information from the subsampling procedure is recorded on the ABL Subsampling Worksheet. Record the following:

1) date
2) actual time required to pick sample (not elapsed time)
3) total number of invertebrates recovered (include extra bugs)
4) number of grids possible on 20 grid subdivided tray (N.B.- the number of grids possible may differ from 20 for various reasons. For example, not enough sample may be present to

cover the entire bottom of the tray, or if quarter grids are processed out of twenty full grids, then a total of 80 grids are possible.

Example: # whole grids X 1 =
half grids X 0.5 = TOTAL GRIDS PROCESSED
quarter grids X 0.25 =

5) the number and size of grids processed (full, half, quarter, etc.) on the 20 grid subdivided tray.

Sorting Procedure

Transfer the contents of a picked sample vial containing 300 organisms into a petri dish. Sort the invertebrates into taxonomic groups (usually to order for insects, but the taxonomic rank to which various non-insect groups are sorted varies). The major groups are listed on the sorting worksheet. One easy way is to remove all specimens of the most common taxon, then move on to the next most common taxon, etc. Place each different taxon in a 1 dram shell vial with 70% ethanol/ 3% glycerol solution; include a correct location and taxon label. This step should be done carefully so that taxa are not mixed. If any invertebrates are encountered that match descriptions above, discard them and record the number discarded on the sorting worksheet. All vials containing sorted taxa from a given site should be bundled together for identification.

Identification

Specimens are identified to the lowest possible taxonomic rank using appropriate taxonomic keys (see CAMLnet document titled "List of California Macroinvertebrate Taxa and Standard Taxonomic Effort"). The number of specimens in each taxon (usually genus for insects) is counted with a laboratory counter, and the results are recorded directly into a computer file (California EDAS, a Microsoft Access© database). If any specimens are discarded (see reasons for discarding specimens above), the number of discards should be recorded per sample on an ABL discard worksheet (see attached).

Each taxon (e.g., genus) within a sample is placed in a separate vial containing a locality label and a taxonomic identification label that includes order, family, genus, number of specimens, and name of taxonomist (see below). Coleopteran larvae and adults are placed in separate vials and recorded separately into CAL EDAS. An organism that cannot be identified to standard taxonomic rank should be recorded as "Undetermined" to the rank of family, order, etc. Identified samples are placed in the WPCL Sample Repository.

Organism Recovery - During the sorting and identification process organisms may be lost, miscounted or discarded. Taxonomists will record the number of organisms discarded and a justification for discarding on the laboratory benchsheets. Organisms may be discarded for several reasons including: 1) subsampler mistakes (e.g. inclusion of terrestrial or semi-aquatic organisms or exuviae), 2) small size (< 0.5 mm), 3) poor condition or 4) fragments of organisms. The number of organisms recovered at the end of sample processing will also be recorded and a percent recovery determined for all samples. Concern is warranted when organism recoveries fall below 90%. Samples with recoveries below 90% should be checked for counting errors and laboratory benchsheets should be checked to determine the number of discarded organisms. If the number of discarded organisms is high, then the technician that performed the subsampling should be informed and re-trained if necessary.

Metrics: The ABL uses a combination of basic descriptive statistics, often referred to as biological metrics, in combination with physical habitat and water chemistry data, to assess the biological integrity of BMI communities. The following table lists the metrics used to describe these communities. Also listed is the expected response of the various measures to impairment.

BMI Metric	Description	Response to Impairment
Richness Measures		
Taxa Richness	Total number of individual taxa	Decrease
Cumulative Taxa	Total number of cumulative taxa	Decrease
Ephemeroptera Taxa	Number of mayfly taxa (genus or species)	Decrease
Plecoptera Taxa	Number of stonefly taxa (genus or species)	Decrease
Trichoptera Taxa	Number of caddisfly taxa (genus or species)	Decrease
EPT Taxa	Number of taxa in the Ephemeroptera (mayfly), Plecoptera (stonefly) and Trichoptera (caddisfly) insect orders	Decrease
Cumulative EPT Taxa (%)	Number of cumulative taxa in the Ephemeroptera (mayfly), Plecoptera (stonefly) and Trichoptera (caddisfly) insect orders	Decrease
Dipteran Taxa	Number of "true" fly taxa, which excludes midges	Increase
Non-Insect Taxa	Number of non-insect taxa	Increase
Composition Measures		
EPT Index (%)	Percent composition of mayfly, stonefly and caddisfly larvae	Decrease
Sensitive EPT Index (%)	Percent composition of mayfly, stonefly and caddisfly larvae with tolerance values between 0 and 3	Decrease
Percent Baetidae	Percent composition of mayfly family nymphs	Decrease
Percent Chironomidae	Percent composition of midge larvae	Increase
Percent Hydropsychidae	Percent composition of caddisfly family nymphs	Decrease
Percent Diptera	Percent composition of "true" fly larvae, which excludes midges	Decrease
Percent Non-insect Taxa	Percent composition of non-insect taxa	Increase
Shannon Diversity Index	General measure of sample diversity that incorporates richness and evenness (Shannon and Weaver 1963)	Decrease
Tolerance/Intolerance Measures		
Tolerance Value (TV)	Value between 0 and 10 weighted for abundance of individuals designated as pollution tolerant (higher values) or intolerant (lower values)	Increase
Percent Intolerant Organisms	Percent of organisms in sample that are highly intolerant to impairment as indicated by a tolerance value of 0, 1 or 2	Decrease
Percent Tolerant Organisms	Percent of organisms in sample that are highly tolerant to impairment as indicated by a tolerance value of 8, 9 or 10	Increase
Percent Dominant Taxon	Percent composition of the single most abundant taxon	Increase
Functional Feeding Groups (FFG)		
Percent Collectors	Percent of macrobenthos that collect or gather fine particulate matter	Increase
Percent Filterers	Percent of macrobenthos that filter fine particulate matter	Increase

Metrics Table (continued)

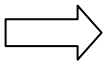
Percent Grazers	Percent of macrobenthos that graze upon periphyton	Variable
Percent Predators	Percent of macrobenthos that feed on other organisms	Variable
Percent Shredders	Percent of macrobenthos that shreds coarse particulate matter	Decrease
	Abundance	
Abundance (#/ sample)	Estimated number of BMIs in sample calculated by extrapolating from the proportion of organisms counted in the subsample	Variable

Taxonomic Reference Collection: A taxonomic reference collection has been established for use in identification of invertebrates. Reference specimens of most taxa are currently available. Specimens of newly encountered taxa should be added whenever possible. When a new taxon is identified, the specimen(s) should be pulled from the bulk samples, labeled and placed in the reference cabinet. The taxon will be entered into CAL EDAS as usual, and it will be indicated that those specimens have been placed in the reference collection.

Labels

Locality Labels: The standard format for location labels is outlined above. This format is used for labeling **all** vials that contain specimens identified to lowest taxonomic rank. In some cases, the full name of the sampling site may be too lengthy to fit on a label of appropriate width. In such cases, the abbreviated site description may replace the full site name. For example:

CA Tehama Co. T1
Dry Creek at Stewart Rd.
ML-3519 M.D., C.S. 10/19/99



CA Tehama Co. T1
Dry Creek DC-SR
ML-3519 M.D., C.S. 10/19/99

Labels are created in Word Perfect. Current labels are stored on the networked lab computers under C: My Documents/Labels. For consistency use the following setup:
From the Word Perfect menu select “Format” “Columns”
In the “Number of Columns” box enter “5”
In the “Space Between” box enter “0.100”
This set up, with a font size of 6, will work for most location labels.

For any given project, it is the responsibility of the person(s) picking the samples to make a batch of labels for that project. Each site within a project has a corresponding column of labels. Labels should be kept in a common-access computer file so that all lab personnel have access to them at all times.

Taxa Labels: In addition to a locality label, each vial of specimens identified to lowest taxonomic rank contains an identification label. The label can be hand-written or pre-printed depending on the preference of the taxonomist. The following is an example of a pre-printed label:

EPHEMEROPTERA
Ephemerellidae
Attenella sp.
det. D. Post

The number of organisms contained in the vial should be hand-written on the label by the taxonomist.

Sample Storage

Original Sample

After a BMI sample has been subsampled the remaining sample is returned to a mason jar with its label, and is kept in storage at the Chico State Lab Facility. The original sample jars are stored in large plastic containers, each of which has a unique number. A storage log has been established that lists the contents of each numbered container so that original samples may be easily recovered.

Original samples are kept until the contracting agency has received the final bioassessment report as specified by an existing contract. Once the final report is delivered, the original samples are returned to the contracting agency at their expense. If the contracting agency does not want the samples, they are disposed of as hazardous waste at the expense of the contractor.

Subsample Remnants

Remnants from the subsampling process are stored only until remnant QC procedures have been completed (see below), after which the subsampled remnants may be discarded.

QUALITY CONTROL REQUIREMENTS

QA for Collecting BMIs

The CSBP is designed to produce consistent, random samples of BMIs. It is important to prevent bias in riffle choice and transect placement. The following procedures will help field crews collect unbiased and consistent BMI samples:

1. In using the CSBP, most sampling reaches should contain riffles that are at least 10 meters long, one meter wide and have a homogenous gravel/cobble substrate with swift water velocity. **There are approved modifications of the CSBP when these conditions do not exist. Contact DFG or visit the California Aquatic Bioassessment Web Site for methods to sample narrow streams, wadeable streams with muddy bottoms and channelized streams.**

2. A DFG biologists or project supervisor should train field crews in the use of the BMI sampling procedures described in the CSBP. Field personnel should review the CSBPs before each field season.

3. During the training, crew members should practice collecting BMI samples as described in the CSBP. The 2 ft^2 area upstream of the sampling device should be delineated using the measuring tape or a metal grid and the collection effort should be timed. Practice repeatedly until each crew member has demonstrated sampling consistency. Throughout the sampling season, assure that effort and sampling area remain consistent by timing sampling effort and measuring sampled area for approximately 20% of the sampling events. The results should be discussed immediately and need not be reported.

QA for Measuring Physical/Habitat Quality

Physical/habitat parameters are assessed using a ranking system ranging from optimal to poor condition. This rapid ranking system relies on visual evaluation and is inherently subjective. The following procedures will help to standardize individual observations to reduce differences in scores:

1. A DFG biologist or a project supervisor should train field crews in the use of the EPA physical/habitat assessment procedures. Field personnel should review these procedures before each field season.

2. At the beginning of each field season, all crew members should conduct a physical/habitat assessment of two practice stream reaches. Assess the first stream reach as a team and discuss in detail each of the 10 physical/habitat parameters described in the EPA procedure. Assess the second stream reach individually and when members are finished, discuss the 10 parameters and resolve discrepancies.

3. Crews or individuals assessing physical/habitat quality should frequently mix personnel or alternate assessment responsibilities. At the end of each field day, crew members should discuss habitat assessment results and resolve discrepancies.

4. The Project Supervisor should randomly pre-select 10 - 20% of the stream reaches where each crew member will be asked to assess the physical/habitat parameters separately. The discrepancies in individual crew member scores should be discussed and resolved with the Project Supervisor.

Analytical Quality Control

Internal QC is conducted by ABL taxonomists on samples that have been processed by the ABL itself. Internal QC procedures target two specific stages of sample processing: the subsampling ("picking") stage and the identification stage.

Subsampling QA (Remnant Evaluation): All remnant samples from every project are examined by a QC taxonomist at the time subsampling is completed. These samples are examined for organisms that may have been overlooked during subsampling. The number of unpicked BMI's (if any) and their identity is recorded in the ABL Quality Control Worksheet. For subsamples containing 300 or more organisms, the remnant sample should contain fewer than 10% of the total organisms subsampled. The remnant should contain fewer than 30 organisms for samples containing fewer than 300 organisms. IF these criteria are not met, then corrective action is initiated. For example, student pickers are currently paid on a per sample basis, which means that they earn more per hour if they process samples quickly. Error rates greater than 10% result in a student earning minimum wage for the time spent processing that sample (or samples).

Internal Taxonomic Identification QA: Taxonomic identifications are evaluated by the ABL's QC taxonomist with the goal of checking the accuracy and consistency of individual taxonomists. Ten percent of the samples from any given project are randomly selected and then checked for taxonomic accuracy. All taxa from each of the randomly selected samples are re-identified by the QC taxonomist, and the number of specimens in each vial is re-checked. Any errors in taxonomy, including misidentification, multiple taxa per vial, counting error and deviation from standard taxonomic effort are recorded in spreadsheet form, and then are analyzed with QC MANAGER, an ACCESS© program that summarizes the types of discrepancy and their frequencies. If a taxonomist is discovered to consistently misidentify a particular taxon, that person will receive instruction from the QC taxonomist about how to properly identify specimens in that group, and all future ID's involving that taxon will be checked until the problem is resolved.

External Quality Control

The ABL has the option of sending all processed samples to an independently contracted lab for external QA/QC of identified specimens. When external QC is performed, 10 percent of all samples are evaluated for taxonomic accuracy and accuracy of specimen counts.

Contract QA

The ABL is sometimes contracted to perform external QA/QC for other independent labs. The protocol outlined above, where 10% of total samples are evaluated for accuracy of taxonomic identifications and specimen counts, is generally followed. QC identifications and counts are compared with the original identifications, any discrepancies are checked to verify that the ABL is not responsible for the error, and a final report is sent to the contracting agency.

INSTRUMENT/EQUIPMENT TESTING, INSPECTION AND MAINTENANCE REQUIREMENTS

The following field equipment and inspection schedule is required for biological sampling:

Equipment Item	Inspection Schedule
D-shaped Kick Net (0.5mm mesh)	Prior to each sampling event
Standard Size 35 Sieve (0.5 mm)	Prior to each sampling event
Wide-mouth Plastic Jars	Prior to each sampling event
Measuring Tape (100 meter)	Prior to each sampling event
Pencils/Permanent Markers	Prior to each sampling event
Flagging	Prior to each sampling event
Forceps	Prior to each sampling event
Water-proof Paper	Prior to each sampling event
Gridded White Enameled Pan	Prior to each sampling event
YSI-85 Meter	Prior to each sampling event
pH Meter	Prior to each sampling event
Thermometer	Prior to each sampling event
Flow Meter	Prior to each sampling event
GPS Unit	Prior to each sampling event
Digital Camera	Prior to each sampling event
Stadia Rod	Prior to each sampling event

INSTRUMENT CALIBRATION AND FREQUENCY

The primary field instruments that require regular calibration are the Oakton brand pHTestr 2 Waterproof Pocket ph Meter and the YSI Model 85 (Dissolved Oxygen, Conductivity, Salinity, and Temperature) Unit. The pH meter used in field surveys involves a two point calibration (pH 7.0 and 10.0), and the YSI Model 85 requires calibration for conductivity against standard solutions (1000 microsiemens +/- 1% at 25 degrees C) and for Dissolved Oxygen on a per use basis. These instruments are calibrated at the beginning of each field season and at 2-3 week intervals thereafter. Prior to taking measurements with these instruments in the field, they are allowed to equilibrate for 15 minutes. **Note: the YSI must be calibrated for Dissolved Oxygen before each use if the elevation changes significantly between sampling sites.**

DATA DEVELOPMENT AND ANALYSIS

The Aquatic Bioassessment Lab is developing Indexes of Biotic Integrity (IBI's) on an ecoregion basis following the methodology of Karr et al. (1986). All methods that employ an IBI to infer water quality ultimately depend on the characterization of regional reference conditions; the ABL is actively pursuing this aspect of project design. In addition, methods for multivariate analysis of benthic communities in relation to local and regional landscape variables are being developed. More information will be presented when standardizes techniques for California become available.

Currently, a taxonomic list of the BMIs identified for each sample is generated for each project along with a summary table of the biological metrics listed on page 17. Metrics are calculated for each sample, and mean values are calculated for each reach. Cumulative values are calculated for richness metrics (total taxa and EPT taxa).Variability of the sample values are expressed as the coefficient of variability (CV). Significance testing can be used for point source sampling programs, and ranking procedures can be used to compare sites sampled using the non-point sampling design (contact DFG for information on ranking formulas), although this method is not an appropriate substitute for an IBI.

Taxa lists and summary metrics are automatically calculated for each project by the Access© database program CAL EDAS. Automation essentially eliminates the chance of human error in metrics calculation. Nonetheless, each set of data is "spot checked" for accuracy by randomly choosing a small subset of metrics and re-calculating those values by hand.

CSBP Stream Habitat Characterization Form

Project Name:		Date:	
Stream Name:		Time:	
Site Code:		Crew Members	
GPS Latitude: ºN			
GPS Longitude: ºW			

SECTION 1. REACH-WIDE PHYSICAL HABITAT SCORES (scores are based on overall reach characteristics and range between 0-20. See EPA's RBP habitat scoring guide for detailed scoring guidelines)

HABITAT MEASURE		SCORE	COMMENTS	TOTAL P-HAB SCORE:
Epifaunal Substrate				
Embeddedness				
Velocity/ Depth Regimes				
Sediment Deposition				
Channel Flow				
Channel Alteration				
Riffle Frequency				
Bank Vegetation	Left Bank	Right Bank		
Bank Stability	Left Bank	Right Bank		
Riparian Zone	Left Bank	Right Bank		

SECTION 2. TRANSECT-SCALE PHYSICAL HABITAT CHARACTERISTICS (measures relate to individual riffles or transects from which each replicate sample is taken**)**

	T1	T2	T3		T1	T2	T3			T1	T2	T3
Average Depth (cm)				Riffle Length (m)				Substrate Composition (percentage composition measured along transect)	Fines (<0.1″)			
Average Velocity (m/s)				Riffle Width (m)					Gravel (0.1-2″)			
Riffle Embeddedness (0-20 scale)				Canopy Cover (%)					Cobble (2-10″)			
Substrate Consolidation (low, med, high)				Substrate Complexity (0-20 scale)					Boulder (>10″)			
Riffle Gradient (this should be recorded as % slope (rise/ run), **not** degrees of slope or inches of drop)									Bedrock (solid)			

SECTION 3. CHEMICAL CHARACTERISTICS (one record per site)

Specific Conductance (µmhos/cm@25ºC)		pH	
Water Temperature (ºC)		Salinity (ppt)	
DO (mg/L)		Alkalinity	

SECTION 4. REACH PHYSICAL CHARACTERISTICS

Reach Length (m)
Photo Exposures

Habitat Parameters	Categories			
	Optimal	**Suboptimal**	**Marginal**	**Poor**
1. Epifaunal Substrate/ Available Cover	Greater than 70% (50% for low gradient streams) of substrate favorable for epifaunal colonization and fish cover; most favorable is a mix of snags, submerged logs, undercut banks, cobble or other stable habitat and at stage to allow full colonization potential (i.e., logs/snags that are not new fall and not transient).	40-70% (30-50% for low gradient streams) mix of stable habitat; well-suited for full colonization potential; adequate habitat for maintenance of populations; presence of additional substrate in the form of new fall, but not yet prepared for colonization (may rate at high end of scale).	20-40% (10-30% for low gradient streams) mix of stable habitat; habitat availability less than desirable; substrate frequently disturbed or removed.	Less than 20% (10% for low gradient streams) stable habitat; lack of habitat is obvious; substrate unstable or lacking.
SCORE ___	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
2a. Embeddedness	Gravel, cobble, and boulder particles are 0-25% surrounded by fine sediment. Layering of cobble provides diversity of niche space.	Gravel, cobble, and boulder particles are 25-50% surrounded by fine sediment.	Gravel, cobble, and boulder particles are 50-75% surrounded by fine sediment.	Gravel, cobble, and boulder particles are more than 75% surrounded by fine sediment.
SCORE ___	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
2b. Pool Substrate Characterization	Mixture of substrate materials, with gravel and firm sand prevalent; root mats and submerged vegetation common.	Mixture of soft sand, mud, or clay; mud may be dominant; some root mats and submerged vegetation present.	All mud or clay or sand bottom; little or no root mat; no submerged vegetation.	Hard-pan clay or bedrock; no root mat or submerged vegetation.
SCORE ___	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
3a. Velocity/ Depth Regimes	All four velocity/depth regimes present (slow- deep, slow-shallow, fast- deep, fast-shallow).	Only 3 of the 4 regimes present (if fast-shallow is missing, score lower than if missing other regimes).	Only 2 of the 4 habitat regimes present (if fast-shallow or slow-shallow are missing, score low).	Dominated by 1 velocity/ depth regime (usually slow-deep).
SCORE ___	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
3b. Pool Variability	Even mix of large- shallow, large-deep, small-shallow, small- deep pools present.	Majority of pools large-deep; very few shallow.	Shallow pools much more prevalent than deep pools.	Majority of pools small- shallow or pools absent.
SCORE ___	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
4. Sediment Deposition	Little or no enlargement of islands or point bars and less than 5% (<20% for low-gradient streams) of the bottom affected by sediment deposition.	Some new increase in bar formation, mostly from gravel, sand or fine sediment; 5-30% (20-50% for low-gradient) of the bottom affected; slight deposition in pools.	Moderate deposition of new gravel, sand or fine sediment on old and new bars; 30-50% (50-80% for low-gradient) of the bottom affected; sediment deposits at obstructions, constrictions, and bends; moderate deposition of pools prevalent.	Heavy deposits of fine material, increased bar development; more than 50% (80% for low-gradient) of the bottom changing frequently; pools almost absent due to substantial sediment deposition.
SCORE ___	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
5. Channel Flow Status	Water reaches base of both lower banks, and minimal amount of channel substrate is exposed.	Water fills >75% of the available channel; or <25% of channel substrate is exposed.	Water fills 25-75% of the available channel, and/or riffle substrates are mostly exposed.	Very little water in channel and mostly present as standing pools.
SCORE ___	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0

6. Channel Alteration	Channelization or dredging absent or minimal; stream with normal pattern.	Some channelization present, usually in areas of bridge abutments; evidence of past channelization, i.e., dredging, (greater than past 20 yr) may be present, but recent channelization is not present.	Channelization may be extensive; embankments or shoring structures present on both banks; and 40 to 80% of stream reach channelized and disrupted.	Banks shored with gabion or cement; over 80% of the stream reach channelized and disrupted. Instream habitat greatly altered or removed entirely.
SCORE ___	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
7a. Frequency of Riffles (or bends)	Occurrence of riffles relatively frequent; ratio of distance between riffles divided by width of the stream <7:1 (generally 5 to 7); variety of habitat is key. In streams where riffles are continuous, placement of boulders or other large, natural obstruction is important.	Occurrence of riffles infrequent; distance between riffles divided by the width of the stream is between 7 to 15.	Occasional riffle or bend; bottom contours provide some habitat; distance between riffles divided by the width of the stream is between 15 to 25.	Generally all flat water or shallow riffles; poor habitat; distance between riffles divided by the width of the stream is a ratio of >25.
SCORE ___	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
7b. Channel Sinuosity	The bends in the stream increase the stream length 3 to 4 times longer than if it was in a straight line. (Note - channel braiding is considered normal in coastal plains and other low-lying areas. This parameter is not easily rated in these areas.	The bends in the stream increase the stream length 2 to 3 times longer than if it was in a straight line.	The bends in the stream increase the stream length 1 to 2 times longer than if it was in a straight line.	Channel straight; waterway has been channelized for a long distance.
SCORE ___	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
8. Bank Stability (score each bank) Note: determine left of right side by facing downstream	Banks stable; evidence of erosion or bank failure absent or minimal; little potential for future problems. <5% of bank affected.	Moderately stable; infrequent, small areas of erosion mostly healed over. 5-30% of bank in reach has areas of erosion.	Moderately unstable; 30-60% of bank in reach has areas of erosion; high erosion potential during floods.	Unstable; many eroded areas; "raw" areas frequent along straight sections and bends; obvious bank sloughing; 60-100% of bank has erosional scars.
SCORE ___(LB)	Left Bank 10 9	8 7 6	5 4 3	2 1 0
SCORE ___(RB)	Right Bank 10 9	8 7 6	5 4 3	2 1 0
9. Vegetative Protection (score each bank) Note: determine left or right side by facing downstream.	More than 90% of the streambank surfaces and immediate riparian zones covered by native vegetation, including trees, understory shrubs, or nonwoody macrophytes; vegetative disruption through grazing or mowing minimal or not evident; almost all plants allowed to grow naturally.	70-90% of the streambank surfaces covered by native vegetation, but one class of plants is not well-represented; disruption evident but not affecting full plant growth potential to any great extent; more than one- half of the potential plant stubble height remaining.	50-70% of the streambank surfaces covered by vegetation; disruption obvious; patches of bare soil or closely cropped vegetation common; less than one-half of the potential plant stubble height remaining.	Less than 50% of the streambank surfaces covered by vegetation; disruption of streambank vegetation is very high; vegetation has been removed to 5 centimeters or less in average stubble height.
SCORE ___(LB)	Left Bank 10 9	8 7 6	5 4 3	2 1 0
SCORE ___(RB)	Right Bank 10 9	8 7 6	5 4 3	2 1 0
10. Riparian Vegetative Zone Width (score each bank riparian zone)	Width of riparian zone >18 meters; human activities (i.e., parking lots, roadbeds, clear-cuts, lawns, or crops) have not impacted zone.	Width of riparian zone 12-18 meters; human activities have impacted zone only minimally.	Width of riparian zone 6-12 meters; human activities have impacted zone a great deal.	Width of riparian zone <6 meters: little or no riparian vegetation due to human activities.
SCORE ___(LB)	Left Bank 10 9	8 7 6	5 4 3	2 1 0
SCORE ___(RB)	Right Bank 10 9	8 7 6	5 4 3	2 1 0

CHAIN OF CUSTODY RECORD

CDFG Aquatic Bioassessment Laboratory

Sampling Agency: ***Project Name:***

Address/Phone of Project Supervisor: ***Crew Member: (Sign and Date)***

Sample #:	***ABL #:***	***Date Collected:***	***Waterbody:***	***Site Description:***	***# of Jars:***

Relinquished By:

(Sign and Date)	***Received By: (Sign and Date)***	***Sample Location***

Page ____of ____

Instructions for Submitting Benthic Samples to California Fish and Game Aquatic Bioassessment Lab

All samples submitted to the Aquatic Bioassessment Lab (ABL) **must** be accompanied by a complete Chain of Custody form. This form must contain the following information:

1. The project name and the watershed name.

2. The name of the agency that completed the original sampling, the name of that agencies' project advisor, the name of at least one crew member that participated in sampling, and address/telephone numbers for both.

3. **Complete locality information** for each sample, including county where sampling occurred, site description (e.g., Pine Creek at Centerville Road), transect information (T1, T2, etc.), sampling date and name of collector.

4. Total number of samples (and total number of jars if different from total samples due to single samples occupying more than one jar).

5. A list of sample ID numbers (***if*** ID numbers have been assigned by the originating agency; otherwise, ID numbers are assigned to each sample by the ABL.

When samples are delivered to the ABL, the delivering agent will be expected to remain at the lab until a member of the ABL staff verifies that all the samples being delivered are listed on the COC form. Any discrepancies will be noted and resolved at the time of delivery. ABL staff will not sign a COC until all discrepancies are resolved. If samples are delivered in the absence of ABL staff, or without the verification of COC contents by ABL staff, the ABL is not responsible for discrepancies (i.e., samples listed on the COC but not actually delivered, or samples delivered without proper documentation on the COC). **Samples will not be accepted without appropriate COC forms.**

CDFG Aquatic Bioassessment Laboratory Subsampling Worksheet

***Project Name:* ________________________ *Project Code:* _______________ *Object Code:* _______________**

ABL #:				**Date:**					**Technician Name:**											
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
random grid #																				
half /whole grid																				
# per grid																				
cumulative #																				
Grids Picked:			Total Grids:				Total Count:				Time:				Comments:					

ABL #:				**Date:**					**Technician Name:**											
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
random grid #																				
half /whole grid																				
# per grid																				
cumulative #																				
Grids Picked:			Total Grids:				Total Count:				Time:				Comments:					

ABL #:				**Date:**					**Technician Name:**											
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
random grid #																				
half /whole grid																				
# per grid																				
cumulative #																				
Grids Picked:			Total Grids:				Total Count:				Time:				Comments:					

\Datasheets\Lab\Labforms\subsampling form2.wpd

CDFG/ CSU Aquatic Bioassessment Laboratory

Picking-Sorting Worksheet

Project Name:					Project Code:			Collection Date:				
Picking									Sorting			
ABL#	Date	Time	# Bugs	# Extra Bugs	Total Bugs includes extras	# Grids Used	# Grids Processed	Tech. Initials	Date	# Bugs	# Discards	Tech. Initials